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Reproduction and growth in Collembola under laboratory conditions

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With 10 figures

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1. Introduction

Two collembolan species, *Orchesella cincta* (L.) and *Tomocerus minor* (LUBBOCK), have been the subject of extensive research concerning their ecology (summarized by JOOSSE 1981 and 1983). Both species can be found in several forests in the Netherlands and live sympatrically. There are important differences between the species concerning their population dynamics. From a comparative demographical field study, performed by VAN STRAALEN (1985), it was shown that *O. cincta* has a higher fertility and a higher mortality compared with *T. minor*. VAN STRAALEN (1985) argued that the higher fertility of *O. cincta* could be explained by an elevated metabolism as a selective effect of temperature. *O. cincta* lives more superficially and the higher temperature allows the species to have a higher mobility. This increases its risk of being predated (ERNSTING *et al.* 1977).

Reproduction in Collembola is iteroparous, which means that an individual reproduces several times in its lifetime. This is brought about by the alternation of feeding instars and reproductive instars, separated by an ecdysis. Reproductive effort is thus strongly related to the moulting frequency. To analyse various aspects of reproduction in detail, in the present paper, data are presented on moulting cycle and clutch size in both species. Moreover, growth and length-mass relation and the relation between mass of the mother, clutch size and egg viability are investigated. The data are discussed in relation to the population turnover rate of both species.

2. Materials and methods

Individuals of *Orchesella cincta* (LINNÉ) and *Tomocerus minor* (LUBBOCK) were collected from the surface litter layer of a pine plantation on Schiermonnikoog, one of the Dutch Wadden Islands, and in a pine plantation at Roggebotzand near Dronten, the Netherlands. They were kept in circular PVC-boxes (⌀ 5 cm), each box containing a male and a female. The bottom of the rearing boxes was provided with a layer of plaster of Paris (1 cm) and placed on a large central disk of moist plaster of Paris. In this arrangement the humidity conditions in all rearing boxes were identical, and fluctuations in humidity were buffered. The rearing boxes were kept in a climate room with a light regime of 12 L/12 D and a temperature of 20 ($\pm$ 1°) °C. The animals were fed with green algae, growing on small twigs. The boxes were checked for egg production, mortality, moisture, food conditions and pollution by faecal pellets every second day. As soon as eggs were found, the mass of the mother (Wp) was measured. All eggs produced in one reproductive instar were considered to belong to one clutch (c). From the moment the eggs hatched, the offspring (F_1) were separated from their parents and placed in a new rearing box.

To study of density effect on reproduction and growth, the animals were reared individually, in pairs or with 5 individuals per box. All the boxes with the F_1-individuals were checked on the presence of exuviae, and the length was measured daily.

After 40 days the animals were offered randomly chosen spermatophores to determine age at, maturity (t) and size of the first clutch.

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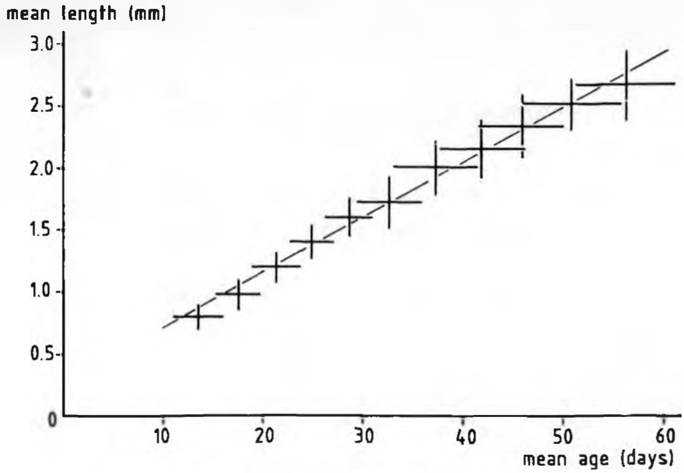


Fig. 1. Relation between mean age (± st. dev.) and mean length (± st. dev.) after moulting in juvenile *O. cincta*.

To measure the size of successive clutches first generation laboratory animals, which had been reared individually, were offered spermatophores throughout their lives. After 95% of the initial experimental group had died, the experiment was terminated. Up to eight successive clutches could thus be scored in *O. cincta* and seven in *T. minor*.

3. Moulting

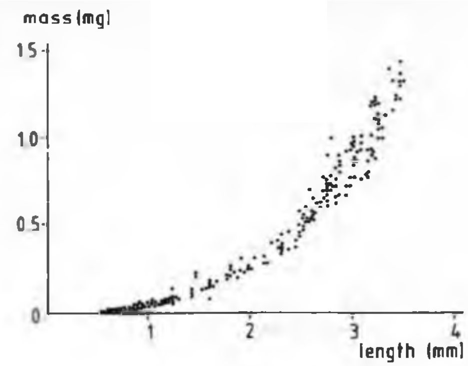
The relation between age and length after each moult in *O. cincta* is presented in Fig. 1. It can be seen that the mean length in individually reared juvenile animals of *O. cincta* shows a linear relation with age. The length of the interval between 2 moults, as shown in Fig. 2, demonstrates a slight increase in time, and the length increase of the individuals per intermoult interval a slight decrease (Fig. 3). This trend continues and is more pronounced in adult animals. With decreasing growth, as shown in the growth curve (Fig. 4), the intermoult interval increases. The mean juvenile instar duration amounts to 4.3 ± 0.6 days in *O. cincta*, which is larger then the value known for *T. minor* (3.7 ± 0.5) as given by Joosse & Veltkamp (1970). Mean adult intermoult intervals were estimated from the data presented in figures 6 and 7. Mean adult intermoult interval for *T. minor* amounted to 7.9 days and for *O. cincta* to 12.1 days.

4. Age, length and number of moults at maturity

4.1. *O. cincta*

Age and length of the individuals and the number of moults at maturity were measured and compared under three different density conditions (D). The data are presented in Table 1a. All data refer to first generation laboratory animals. In the $D = 2$ and $D = 5$ situation the first appearance of eggs of any female was taken as the start of maturity. The data demonstrate no differences in age, length or number of moults at maturity between the three different density conditions, except for age ($P < 0.05$; t-test) and the number of moults at maturity ($P < 0.05$; t-test) between $D = 1$ and $D = 2$.

The correlation and partial correlation coefficients between the age and the length of individuals and the number of moults at maturity is given in Table 1b. It can be noted that there is a significant correlation between length and number of moults (c_{lm}). However, most of this correlation is due to age ($c_{lm \cdot t}$). The correlation between age and moulting is hardly influenced by length. The number of moults at maturity determines in part the correlation between age and length at maturity. It is therefore concluded that age is an important factor in moulting.



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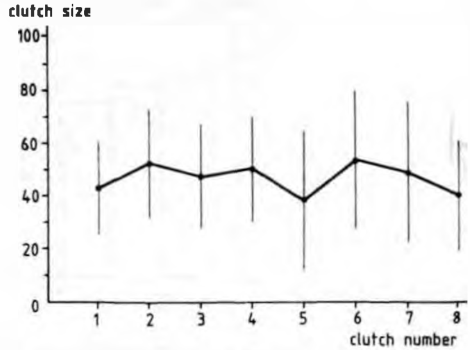

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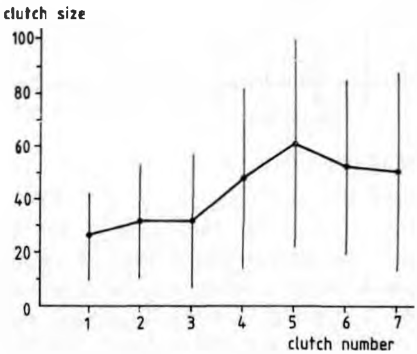
Fig. 5. The relation between length (l) and mass (m) in *O. cincta*: $m = 0.04 \times l^{2.742}$ ($r = 0.99$; $n = 198$).

Fig. 6. The mean and standard deviation of the size of successive clutches in *O. cincta*.



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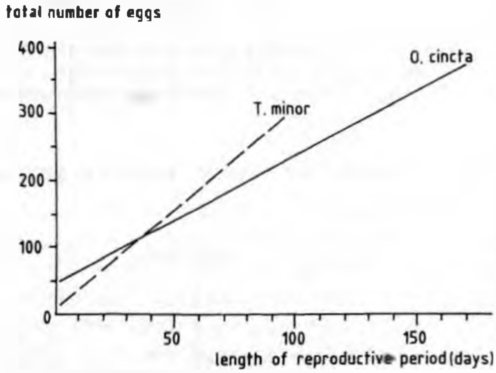

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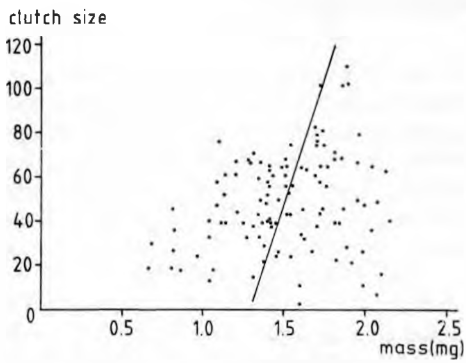
Fig. 7. The mean and standard deviation of the size of successive clutches in *T. minor*.

Fig. 8. Relation between total number of eggs produced and the length of the reproductive period in *O. cincta* (—) and *T. minor* (— —).



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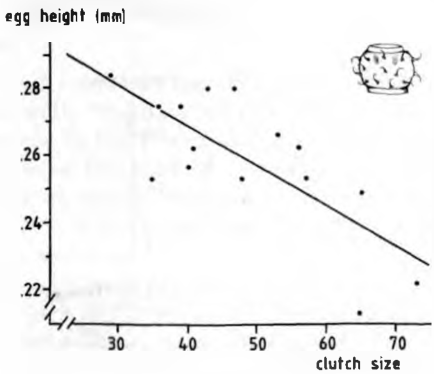

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Fig. 9. Correlation between mass of the mother and the size of her clutch in *O. cincta* ($r = 0.26$; $P < 0.01$; $n = 111$). The principal axis of correlation is shown.

Fig. 10. Correlation between clutch size and height of the eggs in *O. cincta*; $r = -0.76$; $P < 0.01$. The principal axis of correlation is shown.

Table 1a. Mean number and stand-dev. of age (t) and length (l) and number of moults (m) at maturity in *O. cincta* reared under three density conditions: individual (D = 1), in pairs (D = 2) or with five individuals per box (D = 5)

mean ± SD	D = 1	D = 2	D = 5
t (days)	57.0 ± 10.7	67.5 ± 10.3	60.0 ± 9.0
m	11.2 ± 2.0	12.9 ± 1.9	11.4 ± 2.2
l (mm)	2.75 ± 0.31	2.91 ± 0.27	2.72 ± 0.33
	n = 26	n = 13	n = 14

Table 1b. Phenotypic correlation (r) and partial correlation (r′) between age (t), length (l) and number of moults (m) at maturity in *O. cincta*, reared under three density conditions

	D = 1 r/r′	D = 2 r/r′	D = 5 r/r′
tm. l	0.88**/0.77**	0.91**/0.84**	0.81**/0.67*
tl. m	0.76**/0.48*	0.79**/0.60*	0.68**/0.37
lm. t	0.67**/0.01	0.66**/0.22	0.63* /0.19

* $P < 0.05$; ** $P < 0.01$

4.2. *T. minor*

To determine the age and mass at maturity in *T. minor*, juveniles were reared in pairs The design is thus comparable to the D = 2 situation in *O. cincta*. The mean age at maturity amounted to 62.0 ± 9.3 days (n = 66) which is approximately the same as in *O. cincta*. The mean mass at maturity amounted to 0.979 ± 0.29 mg in *T. minor*, which is large compared to the mass at the same age in *O. cincta*, under the same conditions and calculated according to the length-mass relation (0.77 mg). It is concluded that *T. minor* not only moults more frequently during the juvenile phase (see former section) but also grows faster per unit of time compared with *O. cincta*.

5. Growth in *O. cincta*

The relation between age and length of individuals of *O. cincta* forms the growth curve, which is shown in Fig. 4. It is composed from data of two different types of observations. The data in the shaded area of the curve were obtained from individual juvenile animals which were followed from hatching until laying their first clutch. The other data were obtained from individual adult females which were followed from their first up to their last clutch inclusive. In the juvenile only the length was determined, while in the adults only the mass was determined. The length and mass data were converted to mass and length data respectively via the length-mass relation as presented in Fig. 5. The mass-age growth curve (Fig. 4) is S-shaped. Juveniles show exponential growth. The adult growth is slowing down in time towards an asymptote. The asymptotic value of maximum mass was calculated by taking the upper level of the 5% confidence limits of mean calculated from all mass values above $W > 2.0$ mg. The maximum weight in *O. cincta*, calculated in this way, amounts to 2.112 mg and is reached after about 230 days. Only very few animals reached this age and mass.

6. Reproduction

The mean number of eggs per reproductive instar laid by individually reared females of *O. cincta* and *T. minor* after uptake of a spermatophore is illustrated in figs. 6 and 7 respectively. The observations on *O. cincta* were terminated after the production of the 8th clutch. Only 5 of the original 44 females, who produced a first clutch, were still alive. On *T. minor* only 7 successive clutches per female could be observed. From the original 65 females, who produced a first clutch, only 9 survived to produce a 7th clutch. In both

Table 2. Phenotypic correlations between mass of the mother (Mp), clutch size (c), mass of the eggs (m-egg) and height of the egg (h-egg) in *O. cincta* (n = 15)

	Mp	c	m-egg
c	0.50	—	—
m-egg	—0.48	—0.68**	—
h-egg	—0.41	—0.76**	0.87**

* $P < 0.05$; ** $P < 0.01$

Table 3. Summary of the differences in reproduction between *O. cincta* and *T. minor*

	T. minor	*O. cincta*
mean instar duration (days)		
— juvenile	3.7*	4.3
— adult	7.9	12.1
mean clutch size	42.8	46.4
total reproductive period (days)	95	170
number of successive clutches	7	8
mean maximum fertility (number of eggs)	299	375
mean total fertility (eggs/day)	3.15	2.18
mean fertility over the first 36.3 days of the reproductive period (eggs/day)	3.24	3.21

(* value from JOOSSE & VELTKAMP 1970)

species large amounts of phenotypic variation emerged; in *O. cincta* clutch size ranged from 1 to 110 eggs, and in *T. minor* from 1 to 129 eggs. Mean egg production per instar in *O. cincta* was constant up to the 8th clutch (Fig. 6). In *T. minor* the mean egg production per instar reflects a trend of increasing mean fertility up to at least the 5th clutch, but obviously with large variations between individuals (Fig. 7).

Mean clutch size for a total of 8 clutches in *O. cincta* amounts to 46.4 eggs and in *T. minor* for a total of 7 clutches to 42.8 eggs under laboratory conditions. The overall fertility of the two species therefore does not seem to be different. The maximum number of eggs laid by one female *O. cincta* was 375 eggs, over a total reproductive period of 170 days. In *T. minor* the total reproductive period amounted to 95 days. During this time a maximum of 299 eggs per female were produced.

Cumulative egg production, calculated from the means of the successive clutch sizes, plotted against the total reproductive period (Fig. 8), indicate that, measured over the first 35 days, the egg production per unit of time is greater for *O. cincta* compared to *T. minor*, but after this period the egg production per unit of time is greatest in *T. minor*.

The correlation between the mass of the mother (WP) and her clutch size (c) is shown in Fig. 9, and shows an increase in clutch size with the mass of the mother.

To study the effect of the mass of the mother and her clutch size on the size of the eggs, both mass and height (see insert Fig. 10) of the eggs were measured. Height (h) and mass (m) of eggs show a positive correlation (see Tab. 2).

The relation between clutch size and height of the eggs is illustrated in Fig. 10. It appears that the increase in clutch size with the mass of the mother has an effect on the dimensions. Increase in clutch size leads to a significant decrease in both mass and height of the eggs (Table 2).

The question arises now, is there a trade-off between clutch size and egg dimensions, or have the smaller sized eggs in larger clutches any consequence for the offspring's viability? Some disadvantage of the smaller sized eggs in large clutches could be expected. A negative correlation between clutch size and percentage egg hatching ($r = 0.22$; $n = 34$), or between clutch size and growth ($r = 0.02$; $n = 15$) could, however, not be shown. Neither

could a significant correlation between clutch size and carbon content of the eggs be demonstrated ($r = -0.25$; $n = 9$). Finally, also a correlation between egg-mass and percentage survival to adulthood ($r = -0.09$; $n = 15$), and between clutch size and survival percentage ($r = 0.03$; $n = 15$) was absent, probably due to the high overall survival of 90%.

The data on growth and reproduction in *O. cincta* and *T. minor*, as presented in this article, are summarized in Table 3.

7. Discussion

Data about the instar duration demonstrated that for both species, moulting was more frequent in the juvenile period (Table 3), e.g. in periods with faster growth (Fig. 3). Growth depends only in part on moulting, as was shown in the partial correlation analysis (Table 1b). It appeared also that moulting was not determined by length, and it is thus a time fixed phenomenon, which could be expected from an endocrine regulated process.

The results of the mean age and length of individuals, and the number of moults at maturity in *O. cincta* (Table 1a) are in agreement with the observations of other authors. Joosse & Veltkamp (1970) estimated the length of the juvenile period in *O. cincta* to be 60 days. The number of moults up to sexual maturity agrees with that found by Lindenmann (1950) who estimated the number of moults between 10 and 12.

The length at maturity in the present study was large compared to the results of the study by Joosse & Veltkamp (1970), who estimated this length to be approximately 2.3 mm. These authors, however, extrapolated the values from data mainly on adult growth.

The present observations showed a maximum length of *O. cincta* of 4.2 mm at the age of 230 days. Joosse & Veltkamp (1970) estimated a maximum size in *O. cincta* of 3.8 mm at an approximate age of 170 days. Mertens & Blancquaert (1980) reported an approximate age of maximum size of 230 days, and a maximal length of 3.7 mm. The observed differences in maximum length cannot be caused by the differences in age at maximum length, because maximum length was already attained at approximately the age of 170 days (Fig. 4). The greater maximum length, as reported in the present paper, could be the result of the improved rearing conditions.

No difference in total mean clutch size between the two species was found, which is in accordance with Testerink (1982). However, there are differences between the two species in the size of successive clutches (Figs. 6 and 7). The mean size in *O. cincta* is high compared with *T. minor*, especially for the first three clutches. As a consequence of a high mortality in the field, more than two or three clutches per female are rare: less than 2.6% of the spring-population and 0.2% of the autumn population of *O. cincta* produces a second clutch (van Straalen, pers. comm.). As a result of the lower mortality in *T. minor*, the percentage of individuals that will be able to produce two or more successive clutches in the field could be slightly higher. Calculated per unit of time, estimated for the first two clutches, (which equals a period of 36.3 days in *O. cincta*) egg production amounted to 3.21 eggs per day. *T. minor* can, in the same period, produce a third clutch and egg production per day amounted to 3.24 eggs per day. The results presented in this paper, therefore, confirm the suggestion of van Straalen (1985). This author also concluded, on the basis of a comparative demographical field study, that *O. cincta* has a higher fertility compared with *T. minor*, balanced by a higher mortality, and that the lower reproductive performance of *T. minor* cannot be explained quantitatively on the basis of a smaller clutch size, or on the basis of a slower moulting cycle. The higher reproductive effort of *O. cincta* is probably realized by a more effective meeting between the sexes, stimulated by aggregation pheromones (Verhoef *et al.* 1977) and/or by the ability of *O. cincta* to synchronize its reproduction (Joosse & Testerink 1977; Janssen 1985).

A positive correlation between the mass of the mother and the size of her clutch was demonstrated in *O. cincta* (Fig. 9 and Table 2). The larger clutches are made up of smaller sized eggs (Fig. 10). In the present study, however, a superiority neither of the larger eggs, nor of the hatchlings from these larger eggs could be demonstrated. But if large eggs from

small clutches are in no way superior to the smaller eggs from large clutches, the interesting question remains, why females do not always lay large clutches with small eggs. Further research on the trade-off between clutch size and egg size and its consequences for the mother's fitness will by necessary.

8. Acknowledgements

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Synopsis: *Original scientific paper*

Janssen, G. M., & E. N. G. Joosse, 1986. Reproduction and growth in Collembola under laboratory conditions. Pedobiologia **30**, 1—8.

Some autecological aspects, moulting, growth and reproduction of *Orchesella cincta* (L.) and *Tomocerus minor* (Lubbock) were analyzed under standard laboratory conditions (20 °C; 12L/12D).

Mean juvenile instar duration in both species was found to be shorter than adult instar duration. These instar durations were longer in *O. cincta* than in *T. minor*.

Mean age at maturity in both species was approximately the same: 67.5 days in *O. cincta* and 62.0 days in *T. minor*. The number of moults up to sexual maturity was 11—13 in *O. cincta*, while the mean length at maturity ranged from 2.7 to 2.9 mm.

The growth curve in *O. cincta* is S-shaped. Juveniles show exponential growth, and adult growth is slowing down in time, towards an asymptote.

Up to eight successive reproductive instars could be determined in *O. cincta*. Mean clutch size was 46.4 eggs and a total maximum number of 375 eggs. In *T. minor*, mean clutch size was 42.8. eggs, with a total maximum of 299 eggs, measured over seven clutches.

In the field, only the first few clutches can be produced due to high mortality. Egg production per unit of time, calculated over this first period of reproduction, was to 3.2 eggs per day for both species. Despite differences in moulting frequency and clutch size between the two species, the mean fertility was then found to be equal. It is therefore argued that the differences in reproductive performance between the two species in the field cannot be explained on the basis of egg production per unit of time, but could be the result of a higher meeting chance and/or an ability of *O. cincta* to synchronize its reproduction.

In *O. cincta* a significant positive relation between female's mass and clutch size was shown, as well as a trade-off between clutch size and egg size. Hatchlings from small-sized eggs did not show any difference in viability from hatchlings from large sized eggs.

Key words: Collembola, reproduction, growth, moulting, trade off.